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## **X chromosome gain in male breast cancer**

Di Oto, Enrico ; Monti, Valentina ; Cucchi, Maria C ; Masetti, Riccardo ; Varga, Zsuzsanna ; Foschini, Maria P

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DOI: <https://doi.org/10.1016/j.humpath.2015.08.008>

Posted at the Zurich Open Repository and Archive, University of Zurich

ZORA URL: <https://doi.org/10.5167/uzh-113653>

Journal Article

Accepted Version



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Originally published at:

Di Oto, Enrico; Monti, Valentina; Cucchi, Maria C; Masetti, Riccardo; Varga, Zsuzsanna; Foschini, Maria P (2015). X chromosome gain in male breast cancer. *Human Pathology*, 46(12):1908-1912.

DOI: <https://doi.org/10.1016/j.humpath.2015.08.008>

# Accepted Manuscript

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PII: S0046-8177(15)00313-5  
DOI: doi: [10.1016/j.humpath.2015.08.008](https://doi.org/10.1016/j.humpath.2015.08.008)  
Reference: YHUPA 3669

To appear in: *Human Pathology*

Received date: 22 June 2015  
Revised date: 7 August 2015  
Accepted date: 16 August 2015



Please cite this article as: Di Oto Enrico, Monti Valentina, Cucchi Maria C., Masetti Riccardo, Varga Zsuzsanna, Foschini Maria P., X chromosome gain in male breast cancer, *Human Pathology* (2015), doi: [10.1016/j.humpath.2015.08.008](https://doi.org/10.1016/j.humpath.2015.08.008)

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**X CHROMOSOME GAIN IN MALE BREAST CANCER**

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**KeyWords:** Male Breast Cancer, Invasive Ductal Carcinoma, X Chromosome, Genetic marker, FISH.

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## Summary

Male breast cancer (MBC) is an uncommon disease whose molecular profile is not well known. X chromosome gain has been described as a marker of aggressive behavior in female breast cancer. The aim of this study is to investigate the role of the X chromosome in male breast cancer.

20 cases of male breast invasive ductal carcinoma were retrieved and compared with 10 cases of gynecomastia. Cases were tested by fluorescence in situ hybridization (FISH) in order to assess a cytogenetic profile for the X chromosome. The X chromosome status was compared with histopathological features and stage at presentation.

All MBC cases harbored an X chromosome gain (100%) in a variable percentage of neoplastic cells, ranging from 31% to 85% (mean 59%). On the contrary, all cases of gynecomastia showed wild X chromosome asset. The patients' age at surgery and tumor grading showed a statistical significant correlation ( $P= 0,0188$  -  $P= 0,04$ ), with the percentages of neoplastic cells showing an X chromosome gain.

These data suggest that this X chromosome gain plays a role in the neoplastic transformation of male breast epithelial cells.

## 1. Introduction

Male breast cancer (MBC) is an aggressive and uncommon disease accounting for approximately 1% of all breast cancers. The incidence of MBC, once thought to be relatively stable, now seems to be increasing <sup>[1-2-3-4]</sup>. To date, MBC has not been characterized as well as female breast cancer due to its rarity. A lack of specific biomolecular profiles leads clinicians to treat MBC patients in the same way as post-menopausal women <sup>[1-2]</sup>.

Breast cancer is a genetically complex disease characterized by the accumulation of various structural and numerical chromosomal aberrations. In 1964 Jackson <sup>[5]</sup> described an increased risk of breast cancer in male patients with a 47XXY chromosomal asset (Klinefelter's Syndrome), indicating a potential role of X chromosome gain in the neoplastic transformation of the male breast epithelium.

Presently, the role of X chromosome gain has been investigated in prostate carcinoma. Reported data indicate that an additional X chromosome is related to a poor response to anti-androgen therapy and a higher risk of recurrence of prostate carcinoma <sup>[6-8]</sup>.

Little is known about X chromosome gain in breast cancer.

Nakopoulou et. al 2007 <sup>[1]</sup> correlated chromosome X polysomy with a high histological grade, a high nuclear grade and impaired prognosis in cases of invasive ductal carcinoma affecting the female breast. These data suggest an implication of X chromosomes in cell survival and tumor aggressiveness.

On the contrary, no data are presently available on X chromosome gain in MBC.

The purpose of this study was therefore to investigate, using fluorescent in situ hybridization (FISH), the numerical aberrations affecting chromosome X in invasive MBC compared with normal skin as an *in loco* normal control and with gynecomastia. Results on X chromosome numerical aberrations were related to the patients' age, tumor size, and lymph node status at the time of surgery.

## 2. Materials and methods

### 2.1 Patients selection

Twenty consecutive cases of MBC and ten cases of gynecomastia were retrieved from the files of the "M. Malpighi" Anatomic Pathology Section of the Biomedical and Neuromotor Sciences Department of the University of Bologna in Bellaria Hospital.

None of the gynecomastia cases presented MBC.

Cases were retained for the present study when paraffin blocks were available with adequate tissue to allow FISH.

All investigations were conducted according to the principles expressed in the Declaration of Helsinki (Ethical Committee Code: 15004, Prot. N°. 412/CE)

Hematoxylin Eosin -stained slides of all 30 cases were retrieved for histological evaluation, then classified and graded according to currently available criteria <sup>[7-8-9]</sup> by one pathologist (MPF).

All the tissues were fixed in buffered formalin for 24 hours and then paraffin embedded (FFPE) according to routine procedures. FISH was performed on the most representative area of the slide.

## **2.2 Fluorescent In situ Hybridization**

Dual-Color FISH was carried out according to standard FISH protocol, as previously described by Graziano et. al 2011 <sup>[10]</sup>, on 5 µm thick sections of whole tissue using a specific probe mix for the X chromosome (ON AR (Xq12) / SE X, Kreatech Diagnostics, Amsterdam, The Netherlands).

## **2.3 Evaluation Criteria and Data Analysis**

FISH analysis was carried out using an Olympus BX61 epifluorescence microscope (Olympus, Melville, NY) equipped with a 100 planar objective. In each case, at least 100 neoplastic non overlapped nuclei were counted, blindly from clinical information by two different biologists and one pathologist (VM, EDO, MPF).

Normal epithelial tissue adjacent to the neoplastic tissue was used as an internal control to exclude Klinefelter's Syndrome (47XXY).

The following parameters were evaluated in order to correctly interpret the FISH results: total number of X chromosome centromeric probe signals (Spectrum Green), and average number of green signals.

Scoring for X chromosome gain was carried out following criteria by Koivisto et. al (1997)<sup>[11]</sup>. One single signal for both the AR gene (spectrum Orange) and the SE X region (spectrum green) identified the normal chromosome asset. Nuclei with two or more SE X green signals were considered positive for X gain.

Moreover, a lesion containing less than 20% of positive nuclei for X gain was considered to be negative, as described in Ropke et. al 2004 <sup>[6]</sup>.

## 2.4 Statistical Analysis

Statistical analyses were performed using a commercially available software program called QuickCalcs, which is an on-line tool for linear regression by GraphPad.

The relationship between FISH results and clinical and histopathological data was calculated using the generalized Fisher's exact test that is part of the GraphPad QuickCalcs on-line tool.

P values smaller than 0.05 were considered to reflect a significant difference between groups.

## 3. Results

The study included 30 cases, comprising 20 patients with MBC aged from 50 to 81 years (mean 69 years) and 10 patients with gynecomastia aged from 19 to 81 years (mean 58 years). Histological diagnoses of patients with MBC consisted of grade II and grade III invasive ductal breast carcinoma (14/20 cases and 6/20 cases respectively). Metastases to axillary lymph-nodes were present in 4/20 MBC.

All MBC (20/20) showed immunohistochemical positivity for oestrogen receptor (ER), 18/20 for progesterone receptor.

### 3.1 FISH results

The presence of X chromosome gain in more than 20% of the neoplastic cells was detected in 100% (20/20) of the MBC cases studied here (Table 1).

The percentage of neoplastic cells showing X chromosome gain ranged from 31% to 85% (mean 59%)(Fig. 1A-B).

On the contrary, the percentage of cells showing X chromosome gain was below the cut-off value in all the 10 gynecomastia cases (Fig. 1C-D), (Table 2).

No X chromosome gain was observed in epidermal keratinocytes used as an internal control of both the BC and the gynecomastia cases.

### 3.2 FISH profile and clinical-pathological correlation.

A highly significant statistical correlation was found between T size and age in patients with MBC (P value 0.0064) (Fig.2). Age at presentation was related (P value 0.0188) (Fig.3) to the percentage of neoplastic nuclei harbouring X chromosome gain. Furthermore, chromosome X gain was statistically related to the histological grade (P

value 0.04 (Table 3). No statistically significant relationship was found with lymph-node and receptor status at the time of surgery.

#### 4 Discussion

Klinefelter syndrome, characterized by a typical chromosome asset of 47XXY, is the most frequent sex chromosomal disorder in males, accounting for 1\2 per 1000 male neonates <sup>[12]</sup>. It has been demonstrated that Klinefelter syndrome carriers have a higher risk of developing breast cancer<sup>[4,13]</sup>. Although the mechanisms underlying the higher susceptibility to breast cancer in Klinefelter syndrome are not well known, it seems possible that X chromosome gain may play a role in the neoplastic transformation of the male breast epithelial cells.

The mechanism underlying the role of X chromosome in neoplastic transformation is difficult to understand. At present, only data on X chromosome gain regarding prostate cancer are available. The majority of prostate cancers are androgen receptor (AR) dependent. Both AR and its regulator MAGE-11 are mapped on chromosome X, respectively on Xq12 and Xq28, therefore a possible role in recurrence and resistance to anti-androgen therapy has been advocated <sup>[14]</sup>.

The impact of X chromosome gain in female breast cancer has been studied. Original data by Nakapoulou et al <sup>[1]</sup> showed that a gain of the X chromosome was related to an advanced stage at presentation, larger tumour size and high histological grade.

More recently Lin et al <sup>[15]</sup> found aberrant patterns of X-chromosome inactivation in breast cancer cell lines and in breast cancer tissue specimens, significantly related to lower survival rates in female breast cancer patients. Moreover, Muñoz-Rodríguez et al. <sup>[16]</sup> studied the different miRNA expression in female breast cancer appearing immediately after partum or more than 5 years later. These authors found that more than 60% of differentially expressed miRNAs were mapped on the X chromosome.

All these data point toward a possible role of X chromosome involvement in the mechanism of neoplastic epithelial transformation.

To the best of our knowledge, however, the presence of X chromosome gain has not yet been investigated in MBC.

In the present series, all the cases of MBC that were tested showed X chromosome gain in more than 20% of the neoplastic cells.

X chromosome gain was observed also in the gynecomastia cases, even though none of them presented X chromosome gain in more than 20% of the pathological epithelial cells.



Epidemiological data <sup>[17]</sup> show that gynecomastia can be considered a risk factor favouring the occurrence of MBC. The present data, even if limited to a small number of cases, seem to support this hypothesis, suggesting that it represents an early step in the neoplastic transformation of male breast epithelium.

In MBC cases the percentage of neoplastic cells showing X chromosome gain increased in parallel with the age of the patient and the tumour size. The data here shown are consistent with those presented by Jacobs et al <sup>[18]</sup> who demonstrated that sex chromosome aneuploidy in non-dividing nuclei of peripheral blood cells occurs frequently in adult men and increases with age. The data presented by Jacobs et al. <sup>[18]</sup> did not completely clarify the relation between sex chromosome aneuploidy and occurrence of MBC, but suggested a possible role in favouring the neoplastic transformation.

Even though this series is quite small and data should be validated on larger series, possibly completed with follow-up information, they nevertheless indicate that X chromosome gain is an important feature in the early steps of neoplastic transformation of male breast epithelium.

#### **Acknowledgements:**

The study was supported by grants from the Department of Biomedical and Neuromotor Sciences, University of Bologna, Bologna (Italy). VM is supported by the ASAN Foundation ONLUS (Associazione Sostegno e Assistenza Neoplasie, Bologna, Italy).

## References

- [1] Nakopoulou L, Panayotopoulou EG, Giannopoulou I, et al. Extra copies of chromosomes 16 and X in invasive breast carcinomas are related to aggressive phenotype and poor prognosis. *Journal of Clinical Pathology*. 2007;60(7):808-815.
- [2] Harlan LC, Zujewski JA, Goodman MT, Stevens JL. Breast Cancer in Men in the US: A population-based study of diagnosis, treatment and survival. *Cancer*. 2010;116(15):3558-3568.
- [3] Polyak K. Breast cancer: origins and evolution. *The Journal of Clinical Investigation*. 2007;117(11):3155-3163.
- [4] Schottenfeld D, Lilienfeld AM, Diamond H. Some Observations on the Epidemiology of Breast Cancer Among Males. *American Journal of Public Health and the Nations Health*. 1963;53(6):890-897.
- [5] Jackson AW, Muldal S, Ockey CH, O'connor PJ; Carcinoma of Male Breast in Association with the Klinefelter Syndrome, *Brit. Med. J.*, 1965;(1): 223-225.
- [6] Röpke A, Erbersdobler A, Hammerer P, Palisaar, J, John K, Stumm M, Wieacker P, Gain of androgen receptor gene copies in primary prostate cancer due to X chromosome polysomy. *Prostate*, 2004; (59): 59–68.
- [7] Lakhani SR, Ellis IO, Schnitt SJ, Tan PH, van de Vijver MJ. editors. WHO classification of tumours of the breast, World Health Organization classification of tumours (4th ed.) IARC press: Lyon 2012 ISBN 9789283224334.
- [8] Tavassoli F, Eusebi V. editors. AFIP Atlas of Tumor Pathology Series 4, Fascicle 10: Tumors of the Mammary Gland. ARP Press, Silver Spring, Maryland, 2009. ISBN: 9781933477053.
- [9] Elton CW, Ellis IO. Pathological prognostic factors in breast cancer. I. The value of histological grade in breast cancer: experience from a large study with long-term follow-

up. Histopathology. 1991;(5), 403-10.

[10]Graziano F, Galluccio N, Lorenzini P, et al. Genetic activation of the MET pathway and prognosis of patients with high-risk, radically resected gastric cancer. J Clin Oncol 2011; 29,(36), 4789-95,

[11]Koivisto P, Kononen J, Palmberg J, et al., Androgen Receptor Gene Amplification: A Possible Molecular Mechanism for Androgen Deprivation Therapy Failure in Prostate Cancer, Cancer Research 1997; (57), 314-319.

[12]Nieschlag E. Klinefelter Syndrome The Commonest Form of Hypogonadism, but Often Overlooked or Untreated. Dtsch Arztebl Int. 2013; 110(20): 347–53.

[13]Ottesen AM, Garn ID, Aksglaede L, Juul A, Rajpert-De Meyts E. A simple screening method for detection of Klinefelter syndrome and other X-chromosome aneuploidies based on copy number of the androgen receptor gene. Mol Hum Reprod. 2007; (10):745-50.

[14]Wilson EM. Androgen receptor molecular biology and potential targets in prostate cancer. Ther Adv Urol 2010; 2: 105-117.

[15]Lin IH, Chen DT, Chang YF, et al. Hierarchical Clustering of Breast Cancer Methylomes Revealed Differentially Methylated and Expressed Breast Cancer Genes. PLoS One. 2015; 10(2): e0118453.

[16]Muñoz-Rodríguez JL, Vrba L, Futscher BW, et al. Differentially Expressed MicroRNAs in Postpartum Breast Cancer in Hispanic Women. PLoS ONE 2015; 10(4): e0124340.

[17] Brinton LA, Cook MB, McCormack V, et al. Anthropometric and Hormonal Risk Factors for Male Breast Cancer: Male Breast Cancer Pooling Project Results. JNCI J Natl Cancer Inst (2014) 106(3): djt465 doi:10.1093/jnci/djt465.

[18] Jacobs PA, Maloney V, Cooke R, Crolla JA, Ashworth A, Swerdlow AJ. Male

breast cancer, age and sex chromosome aneuploidy. British Journal of Cancer (2013) 108, 959–963.

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**Figure legend:**

Figure legend:

Fig.1.

brief title: FISH results

and a description: (A) H&E-stained section showing invasive ductal carcinoma case and its positivity by FISH analysis (X 1000) for X chromosome gain (the number of green signals indicating the X chromosome are  $> 2$  in the neoplastic nuclei) (B). (C) H&E-stained section showing gynecomastia (X 250) case and its negativity for X chromosome gain by FISH (X 1000) (the number of green signals indicating the X chromosome are  $< 2$  in the pathological nuclei) (D).

Fig.2.

brief title: linear regression

and a description: The figure evidences the correlation between tumor size and age in patients with male breast cancer (P value 0.0064).

Fig.3.

brief title: linear regression

and a description: The figure evidences the correlation between age at presentation and percentage of neoplastic nuclei harboring X chromosome gain (P value 0.0188).

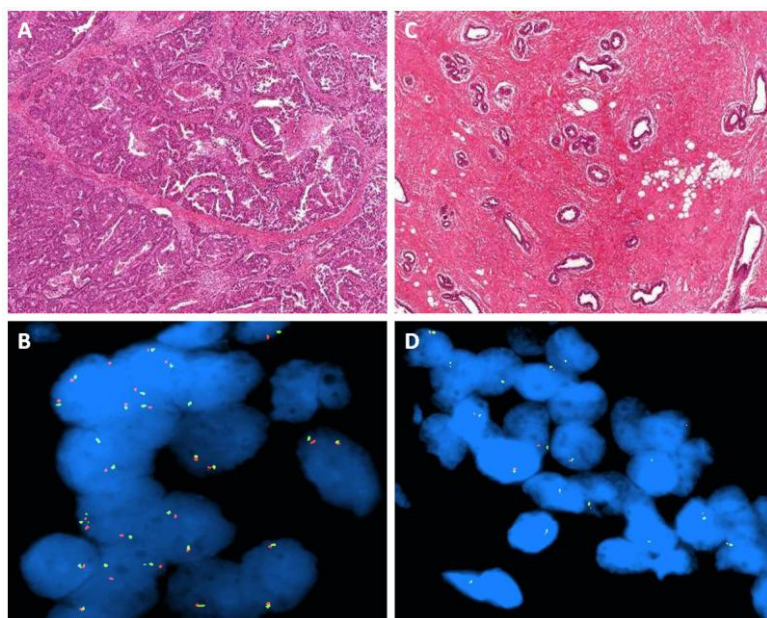


Figure 1

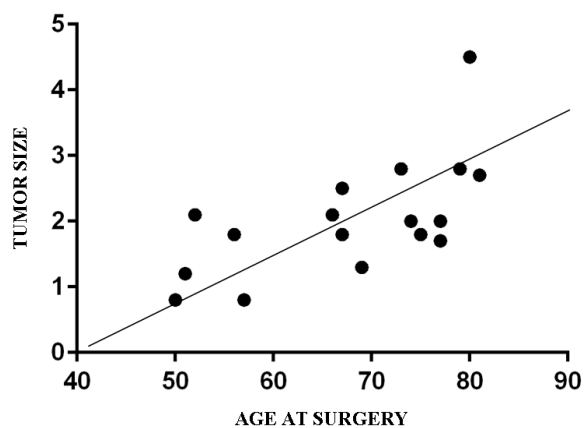


Figure 2

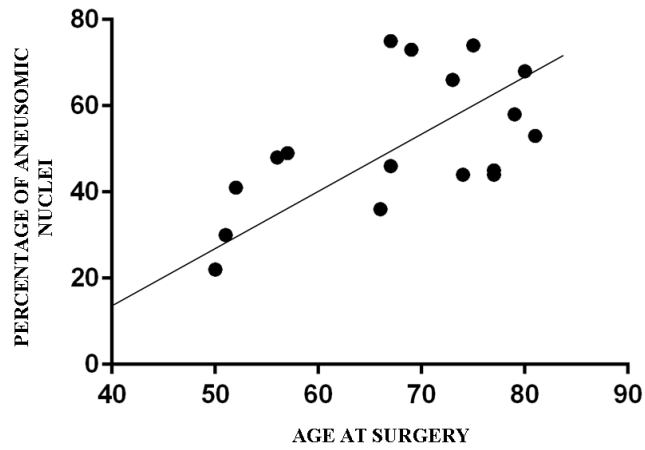


Figure 3



**Table 1.** Summary of clinical-pathological parameters and X chromosome status by FISH

<b>Id</b>	<b>Age at Surgery</b>	<b>X Aneusomy Asset by FISH*</b>	<b>X Aneusomy**</b>	<b>Histological Grade</b>	<b>Tumor size (cm)</b>
<b>1</b>	80	25X, 68XX, 7XXX	68	2	4.5
<b>2</b>	52	52X, 41XX, 3XXX, 4XXXX	41	2	2.1
<b>3</b>	51	69X, 30XX, 1XXX	30	2	1.2
<b>4</b>	69	19X, 73XX, 3XXX, 4XXXX, 1VIX	73	2	1.3
<b>5</b>	79	31X, 58XX, 10XXX, 1XXXX	58	3	2.8
<b>6</b>	81	44X, 53XX, 3 XXX	53	3	2.7
<b>7</b>	56	52X, 48XX	48	2	1.8
<b>8</b>	57	48X, 49XX, 3XXX	49	3	0.8
<b>9</b>	66	64X, 36XX	36	2	2.1
<b>10</b>	77	55X, 44XX, 1XXX	44	2	1.7
<b>11</b>	73	28X, 66XX, 6XXX	66	2	2.8
<b>12</b>	67	51X, 46XX, 2XXX, 1XXXX	46	2	2.5
<b>13</b>	67	15X, 75XX, 5XXX, 5XXXX	75	3	2.3
<b>14</b>	50	21X, 22XX, 28XXX, 23XXXX, 5XXXXX, 1 XXXXXX	22	2	0.8
<b>15</b>	75	24X, 74XX, 2XXX	74	3	2.3
<b>16</b>	77	50X, 46XX, 4XXX	46	2	2
<b>17</b>	74	53X, 44XX, 1XXX, 1XXXX, 1 XXXXXX	44	2	2
<b>18</b>	80	29X, 69XX, 2XXX	69	3	1.3
<b>19</b>	70	55X, 44XX, 2XXX	44	2	2.5
<b>20</b>	74	37X, 60XX, 2XXX, 1XXXX	60	2	2.5

**Legend:**

\*Indicates the general asset of X chromosome observed in 100 nuclei for each MBC.

\* \*The values indicate the number of nuclei (evaluated on 100 nuclei) with 2 X chromosome signals.

**Table 2.** X chromosome status by FISH in patients with gynecomastia

<b>Id</b>	<b>Age at surgery</b>	<b>X Aneusomy Asset by FISH*</b>	<b>Nuclei WT**</b>	<b>N. of X nuclei Aneusomy***</b>
<b>G1</b>	63	100X	100	0
<b>G2</b>	68	90X,10XX	90	10
<b>G3</b>	75	95X,5XX	95	5
<b>G4</b>	65	98X,2XX	98	2
<b>G5</b>	23	95X,5XX	95	5
<b>G6</b>	26	97X,3XX	97	3
<b>G7</b>	81	89X,11XX	89	11
<b>G8</b>	61	94X, 6XX	94	6
<b>G9</b>	24	93X, 7XX	93	7
<b>G10</b>	19	94X, 6XX	94	6

**Legend:**

\*Indicates the general assets of X chromosome observed in 100 nuclei for each case of gynecomastia.

\* \*The values indicate the number of nuclei with normal asset of X chromosome in male (then one X chromosome for nuclei)

\*\*\*Number of nuclei (evaluated in 100 consecutive nuclei) with more than one X chromosome.

**Table 3.** Correlation between X Aneusomy status and Histological Grade

	<b>Grade 2</b>	<b>Grade 3</b>	<b>Total</b>
<b>XX* &gt;50%</b>	4	5	<b>9</b>
<b>XX* &lt;50%</b>	10	1	<b>11</b>
<b>Total</b>	<b>14</b>	<b>6</b>	<b>20</b>

**Legend:**

\*XX indicate the X aneusomy in MBC.